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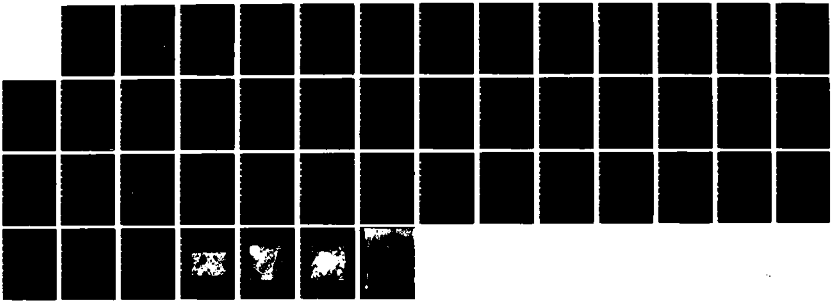

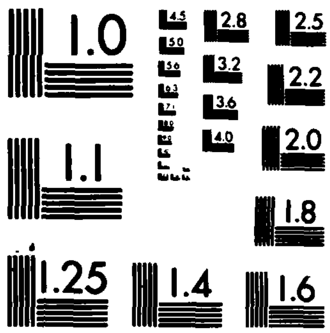

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Mechanisms of Protective Immunogenicity of Microbial Vaccines of Military Medical Significance

Annual Summary Report

Michael S. Ascher, M.D.

30 January 1981

Supported by

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Clinical studies of Q fever vaccine ~~(phase I, IND #610)~~ have been undertaken with screening of volunteers for humoral and cellular immune reactivity to specific Q fever antigens.

A rapid and simplified immunofluorescent procedure has been developed for serodiagnosis of Q fever and tularemia suitable for field use. Excellent correlation exists between the results of this procedure and standard complement fixation testing for diagnosis of Q fever.

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Lot-to-lot variability of phase I Q fever vaccine has been assessed by in vitro lymphocyte transformation testing of immune guinea pigs. There is a consistent response between lots of vaccine by this technique and no evidence of endotoxin contamination in the vaccine

Delayed hypersensitivity skin testing of guinea pigs with Q fever antigen has revealed granulomatous changes with giant cell prominence. The immunologic basis of the granulomatous response is under analysis.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

SUMMARY

Clinical studies of Q fever vaccine (phase I, IND #610) have been undertaken with screening of volunteers for serologic and cellular immune reactivity to Q fever antigen. The results indicate a low level of response in these subjects making them excellent candidates for the vacine trial in progress.

A rapid and simplified immunofluorescent procedure has been developed for serodiagnosis of Q fever and tularemia suitable for field use. Excellent correlation exists between the results of this procedure and standard complement fixation testing.

Lot-to-lot variability of phase I Q fever vaccine has been assessed by *in vitro* lymphocyte transformation testing of immune guinea pigs. There is excellent agreement between lots of vaccine by this technique and no evidence of contamination.

Delayed hypersensitivity testing in guinea pigs with Q fever antigen has revealed features of granulomatous hypersensitivity with epitheloid and giant cell prominence. The immunologic basis of the granulomatous response is under analysis.

PROGRESS

1. Studies of Phase I Q Fever Vaccine in Human Volunteers.

Human studies have been held in abeyance pending final approval by USAMRIID of our revised protocol and consent form which include a skin biopsy of delayed hypersensitivity test sites. In the meantime, volunteers have been recruited and tested for sensitivity to Q fever by immunofluorescence and lymphocyte transformation. There are at present 24 individuals at UCI with experimental contact with sheep who are eligible to participate in our study. In addition, 5 individuals from an Army reserve unit have expressed interest in participation. In terms of personnel, it appears therefore that there will be no difficulty obtaining suitable volunteers when the vaccine is finally released for our study. As alluded to above, we have added a punch biopsy of skin test sites to the protocol because of findings in guinea pigs noted below. This work will be done in collaboration with Dr. Ronald Barr of the Department of Dermatology at UCI. As depicted in Table 1, there is a spectrum of response in these individuals to our panel of ubiquitous microbial antigens (PPD, SKSD and Diptheria toxoid) but in general a low level of baseline lymphocyte reactivity to C. burnetii antigen. This is in agreement with the serologic findings in this population in that all the individuals but #1 are seronegative by complement fixation testing. This one exception carries a titer of 1:8 and has a lymphocyte response which is higher than all the others albeit it at a low level. This observation confirms our previous findings of a high degree of sensitivity of lymphocyte transformation testing in man. These findings also assure us of the feasibility of performing lymphocyte studies on a large scale as required by our vaccine trial. Two practical impediments to the field use of lymphocyte transformation have been addressed this year. First, most LT techniques require freshly obtained white cell-rich plasma for culture. Previously we have shown that if blood is allowed to sit for only one hour, there is a considerable loss of reactivity, most pronounced with specific antigen stimulation. We have now developed a method to overcome this difficulty by adding the sedimenting agent, high molecular weight dextran, after the cells have sat (or travelled) or an hour. As illustrated in Table 2, there is excellent preservation of reactivity using this procedure compared to the other alternative tested. The second impediment relates to the requirement for preparation of fresh microtiter plates for cultures. One possible solution to this problem would was to preload plates with antigen and lyophylize them and at the end of the culture period, the plates could be frozen and held indefinitely prior to harvest. As illustrated in Table 3, neither of these techniques is associated with any appreciable decrease in response of the lymphocytes tested. These two modifications now make LT testing a practical field technique.

2. Serodiagnosis of Q Fever.

Under this contract we have been successful in adapting a rapid immunofluorescence (FA) procedure to the serodiagnosis of Q fever. We have had the opportunity to test a large number of positive samples from recent laboratory associated infections by both standard complement fixation (CF) and FA testing. The full details of the technique and results are documented in the enclosed abstract, book chapter, and draft manuscript. In our hands this procedure is exquisitely sensitive and able to detect changes in guinea pigs exposed to a 60 ng skin test dose of Q fever vaccine. We have also documented that the test results are very consistent on serial samples obtained from the same individual. All these factors should make this assay very useful in studying the serologic response of humans to Q fever vaccine. There was little expectation that CF testing would be useful in vaccinees and this assay may alleviate this problem.

In studies not covered in the enclosed preprints we have used the FIAX procedure to test sera from sheep and find an excellent correlation between it and microagglutination testing performed at UC Davis. These results are presented in Table 4. This feature of the procedure should simplify performing serologic surveys of experimental animals. From the military point of view, rapid screening of indigenous livestock by units in the field would be a useful technique to identify situations with high risk of Q fever. Such a procedure would have been very useful in restrospect in dealing with the problem of Q fever in World War II.

We have also tested FIAX serology in the diagnosis of tularemia with Foshay vaccine as antigen. Table 5 shows the result of a pilot study in one immune and one nonimmune individual. There is excellent separation with this test and the procedure is clearly easier than microagglutination most commonly used elsewhere. It is apparent from the Q and tularemia systems that almost any infectious disease model where relatively purified antigen is available can be adapted to FIAX technology giving the potential of a panel of dipsticks for screening for exotic diseases. The additional features of the assay which emphasize this potential are the stability and transportability of antigen-coated sticks and the lack of requirement for making dilutions of serum in performing the test. These features also make the technique feasible in a field or biohazard setting which is very hard to say about other immunofluorescent technology currently available.

3. In Vitro Cellular Immunologic Studies of Q Fever Vaccine in Guinea Pigs.

Previous work has demonstrated our ability to detect *in vitro* lymphocyte proliferative responses in guinea pigs immunized with Q fever vaccine. We have utilized these techniques in assessing the lot-to-lot variability of Phase I vaccine. Guinea pigs were immunized with 12 ug lot 6 vaccine in Freund's complete adjuvant and LT testing was performed at three times before and after immunization. The results are presented in Figure 1 and

show a remarkable consistency in response between lots of vaccine. The three lines depict the geometric mean response $\pm$ SE of eight animals at each time point. For the sake of emphasis, Figure 2 shows the results of the eight individual animals cultured at week two. There is excellent agreement within animals between lots across a wide range of responses. In the studies performed prior to immunization, there is no mitogenic activity in vaccine which is evidence against endotoxin in any of the lots. The immune guinea pigs respond equally well to all five lots tested throughout the course of their development of immunity. It would seem on the basis of these results that any of the lots of vaccine can be used for in vitro LT testing without undue concern for the issue of lot-to-lot variability.

4. Analysis of Guinea Pig Skin Reactions to Q Fever Vaccine.

Pending the start of human studies, much of the work performed under this contract has focused on definition of the nature of the skin test responses of immunized guinea pigs to Q fever antigen. The literature has amply documented that atypical late reactions are a regular feature of skin testing for Q fever but no biopsy results in man or experimental animals have been reported. We have previously presented data on the time course of development of induration of these tests. Subsequently, we noted that the results are superimposable on those obtained in guinea pigs immunized with sodium zirconium lactate, a model for the study of granulatous hypersensitivity developed by Professor John L. Turk of the Royal College of Surgeons, London, England. Our prior association with Professor Turk led to renewed collaboration and through the Wellcome Foundation, we spent three weeks in London discussing the results of this collaboration. The findings are presented in detail in the attached abstract and draft manuscript. In brief, we note the presence in the skin test sites of classic epitheloid granulomas with large numbers of giant cells. Peculiar to the histology of these reactions is the presence of polymorphonuclear leukocytes leading to the histologic term of "leukocytoclastic changes." This lesion may have special significance in light of the immunopathogenesis of chronic Q fever and will be the subject of further analyses in the renewal of this contract.

One immediate idea that comes to mind in studies of delayed hypersensitivity to Q fever, is the development of a purified nongranulomagenic reagent which could substitute for whole organism antigen in clinical use. One obvious candidate reagent for this purpose would be the so-called soluble antigen obtained after TCA extraction. As shown in Figure 3, when compared to whole vaccine, soluble antigen has relatively high granulomagenic potential but we have not yet tested the residue of the TCA extraction in the skin test assay as a control. Histologic studies of these reactions are also in progress. Further studies in this direction are presented in detail in our renewal proposal.

TABLE 1

HUMAN LYMPHOCYTE PROLIFERATIVE RESPONSES TO A PANEL OF MICROBIAL ANTIGENS

		Antigen added (Concentration)								
							C. BURNETII			
							—PHASE I—		—PHASE II—	
CF Titer	None	TUL (1ug)	PPD (1ug)	DIPH (2.3Lf)	SKSD (20U)	PHA (1:1000)	(1:10)	(1:100)	(1:10)	(1:100)
16	72*	95 (1.3)	732 (10.2)	181 (2.5)	135 (1.9)	2863 (39.8)	803 (11.2)	769 (10.7)	960 (13.3)	718 (10.0)
<4	104	79 (0.8)	250 (2.4)	1646 (15.8)	600 (5.8)	3752 (36.1)	77 (0.7)	92 (0.9)	159 (1.5)	90 (0.9)
<4	96	128 (1.3)	1451 (15.1)	250 (2.6)	2505 (26.1)	2899 (30.2)	95 (1.0)	78 (0.8)	147 (1.5)	84 (0.9)
<4	83	395 (4.8)	908 (10.9)	89 (1.1)	397 (4.8)	4489 (54.1)	81 (1.0)	87 (1.0)	152 (1.8)	82 (1.0)
<4	94	162 (1.7)	491 (5.2)	144 (1.5)	441 (4.7)	2896 (30.8)	137 (1.5)	132 (1.4)	684 (7.3)	266 (2.8)
<4	175	143 (0.8)	308 (1.8)	535 (3.1)	3112 (17.8)	4621 (26.4)	72 (0.4)	83 (0.5)	324 (1.9)	132 (0.8)
<4	57	60 (1.1)	1617 (28.4)	1116 (19.6)	2767 (48.5)	9155 (161)	42 (0.7)	182 (3.2)	78 (1.4)	70 (1.2)
<4	158	135 (0.9)	3502 (22.2)	1027 (6.5)	1518 (9.6)	5478 (34.7)	147 (0.9)	117 (0.7)	257 (1.6)	76 (0.5)
<4	160	2552 (16.0)	1224 (7.6)	470 (2.9)	1433 (9.0)	6172 (38.6)	127 (0.8)	276 (1.7)	264 (1.7)	200 (1.3)
<4	84	175 (2.1)	670 (8.0)	2115 (25.2)	667 (7.9)	4469 (53.2)	95 (1.1)	92 (1.1)	339 (4.0)	108 (1.3)
<4	137	118 (0.9)	121 (0.9)	1373 (10.0)	328 (2.4)	2080 (15.2)	95 (0.7)	106 (0.8)	134 (1.0)	129 (0.9)
<4	110	108 (1.0)	2348 (21.3)	3082 (28.0)	1507 (13.7)	574 (5.2)	90 (0.8)	84 (0.8)	217 (2.0)	115 (1.0)

*Mean CPM/well (Stimulation Index) of four replicates

TABLE 2

EFFECTS OF VARIOUS TREATMENTS ON

HUMAN LYMPHOCYTE PROLIFERATIVE RESPONSES

Treatment of Plate	None	Antigen added (Concentration) TUL (lug)	PPD (lug)	DIPH (2.3Lf)	SKSD (20U)	PHA (1:1000)
None	106	2451 (23.1)	121 (1.1)	156 (1.5)	1223 (11.5)	4587 (43.3)
Preloaded - Frozen	83	2414 (29.1)	169 (2.0)	114 (1.4)	1516 (18.3)	5414 (65.2)
Preloaded - Frozen Lyophilized	57	2056 (36.1)	369 (6.5)	405 (7.1)	1397 (24.5)	5523 (96.9)
Frozen Prior to Harvest	127	2463 (19.4)	225 (1.8)	200 (1.6)	1384 (10.9)	5076 (40.0)

*Mean CPM/well (Stimulation Index) of four replicates

TABLE 3

EFFECTS OF DIFFERENT METHODS OF WHITE CELL SEPARATION ON

HUMAN LYMPHOCYTE PROLIFERATIVE RESPONSES

METHOD OF LEUKOCYTE SEPARATION	None	Antigen added (Concentration) TUL (lug)	PPD (lug)	DIPH (2.3Lf)	SKSD (20U)	PHA (1:1000)
Normal (Immediate Dextran + Sedimentation)	207	2537 (12.3)	159 (0.8)	486 (2.3)	1029 (5.0)	4853 (23.4)
Sit with Dextran 1 hr. Mix - Sediment	64	2473 (38.6)	169 (2.6)	637 (10.0)	798 (12.5)	6087 (95.1)
Sit 1 hr., add Dextran Mix - Sediment	116	3278 (28.3)	317 (2.7)	661 (5.7)	954 (8.2)	4759 (41.0)
Sediment - Aspirate Plasma - Sit 1 hr.	158	2908 (18.4)	328 (2.1)	499 (3.2)	456 (2.9)	5200 (32.9)

*Mean CPM/well (Stimulation Index) of four replicates

TABLE 4

FIAX ASSAY OF ANTIBODY TO PHASE II C. BURNETII IN SHEEP

MA Value	N	FSU (SD)
Neg	3	11.8 (0.5)
8-16	2	52.0 (22.0)
>16	3	77.0 (29.0)

TABLE 5

FIAX ASSAY OF ANTIBODY TO TULAREMIA ANTIGEN

		Serum Dilution		
Antigen Dilution*	Serum Source	1:40	1:160	1:640
1:4	Immune	>200**	161	50
	Nonimmune	91	39	27
1:16	Immune	134	81	29
	Nonimmune	38	14	9
1:64	Immune	42	20	7
	Nonimmune	11	6	2

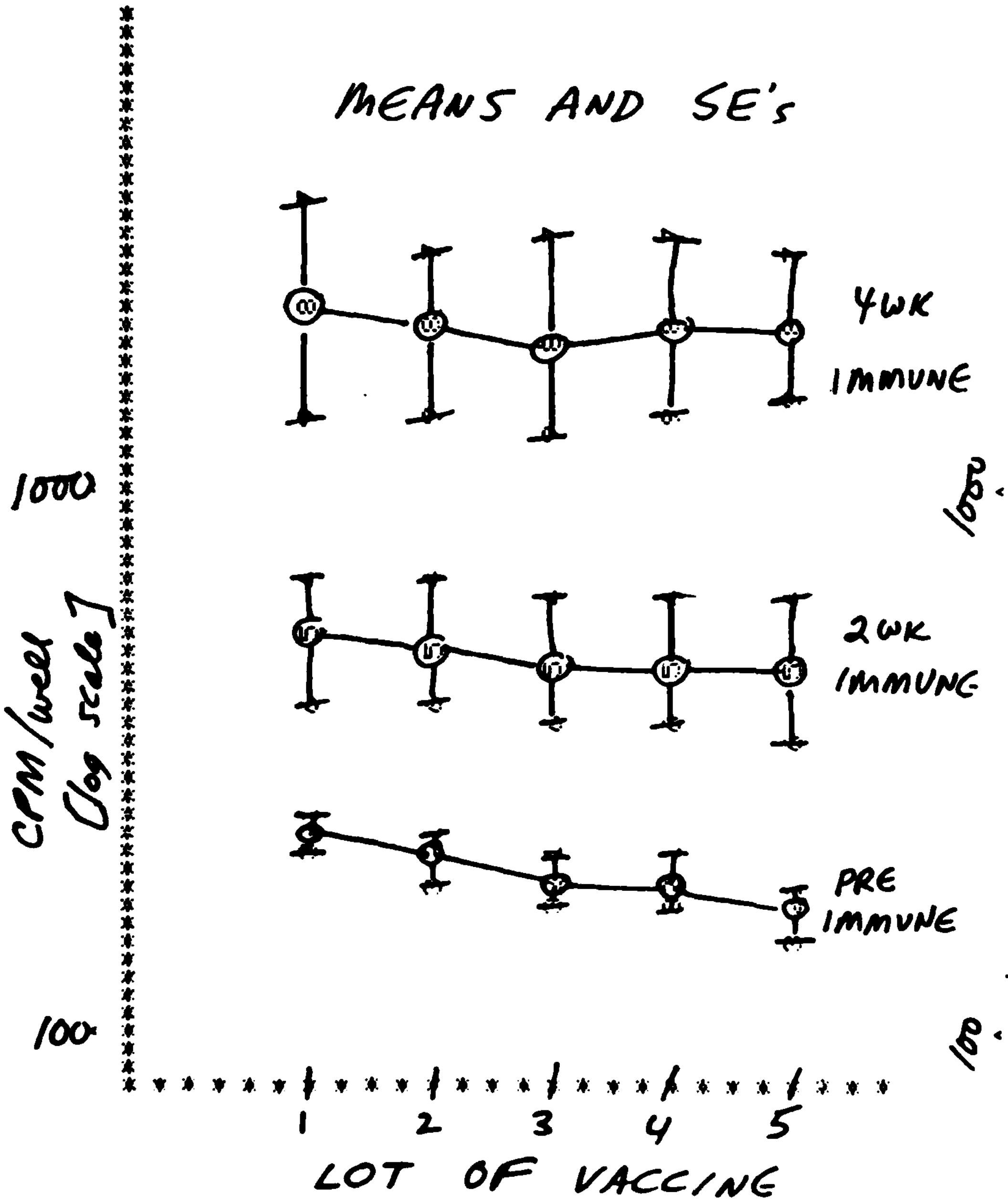
*Foshay skin test antigen reconstituted to 9.8 ug N/ml

**Specific FSU

FIGURE 1

FIGURE 2

LOT-TO-LOT VARIABILITY OF
Q FEVER VACCINE ASSESSED BY LT



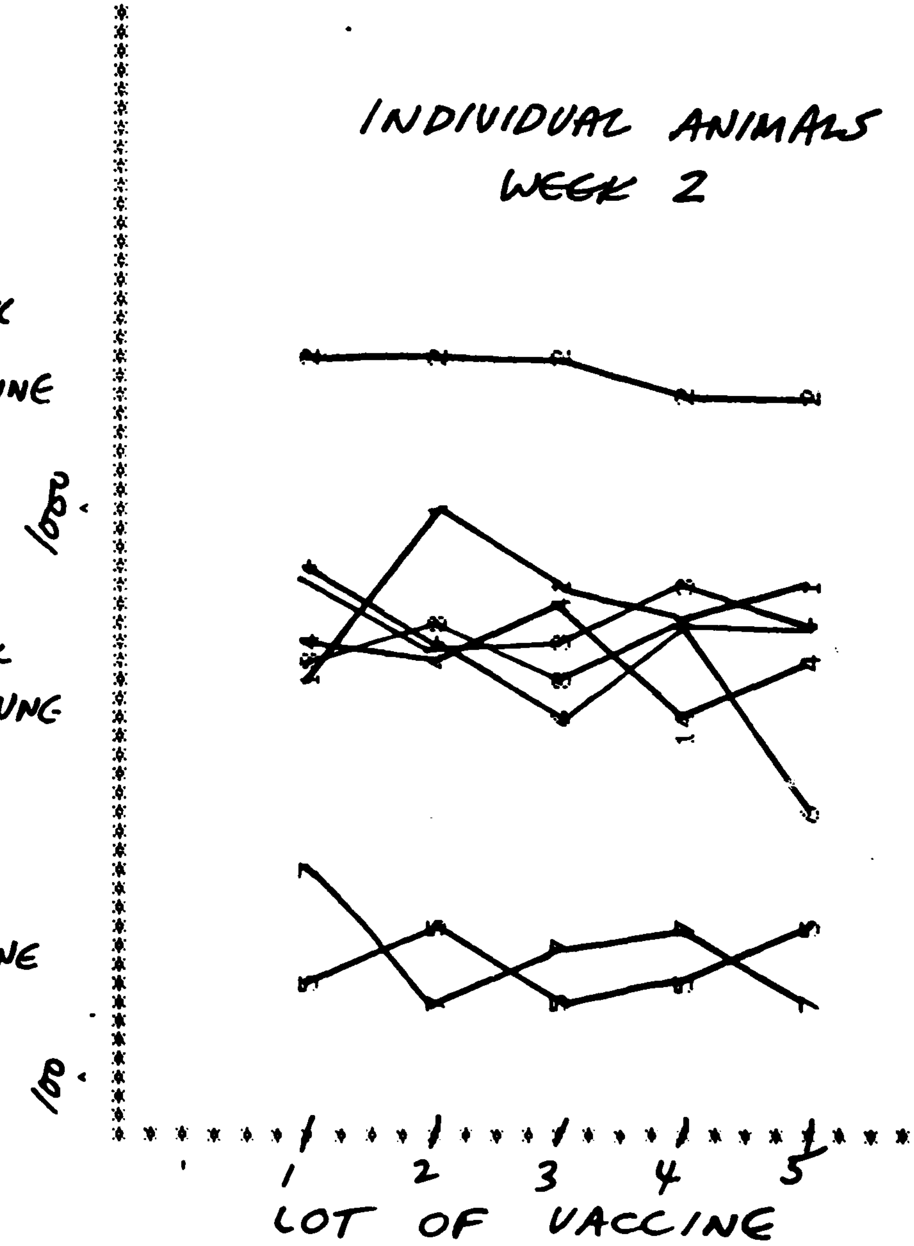
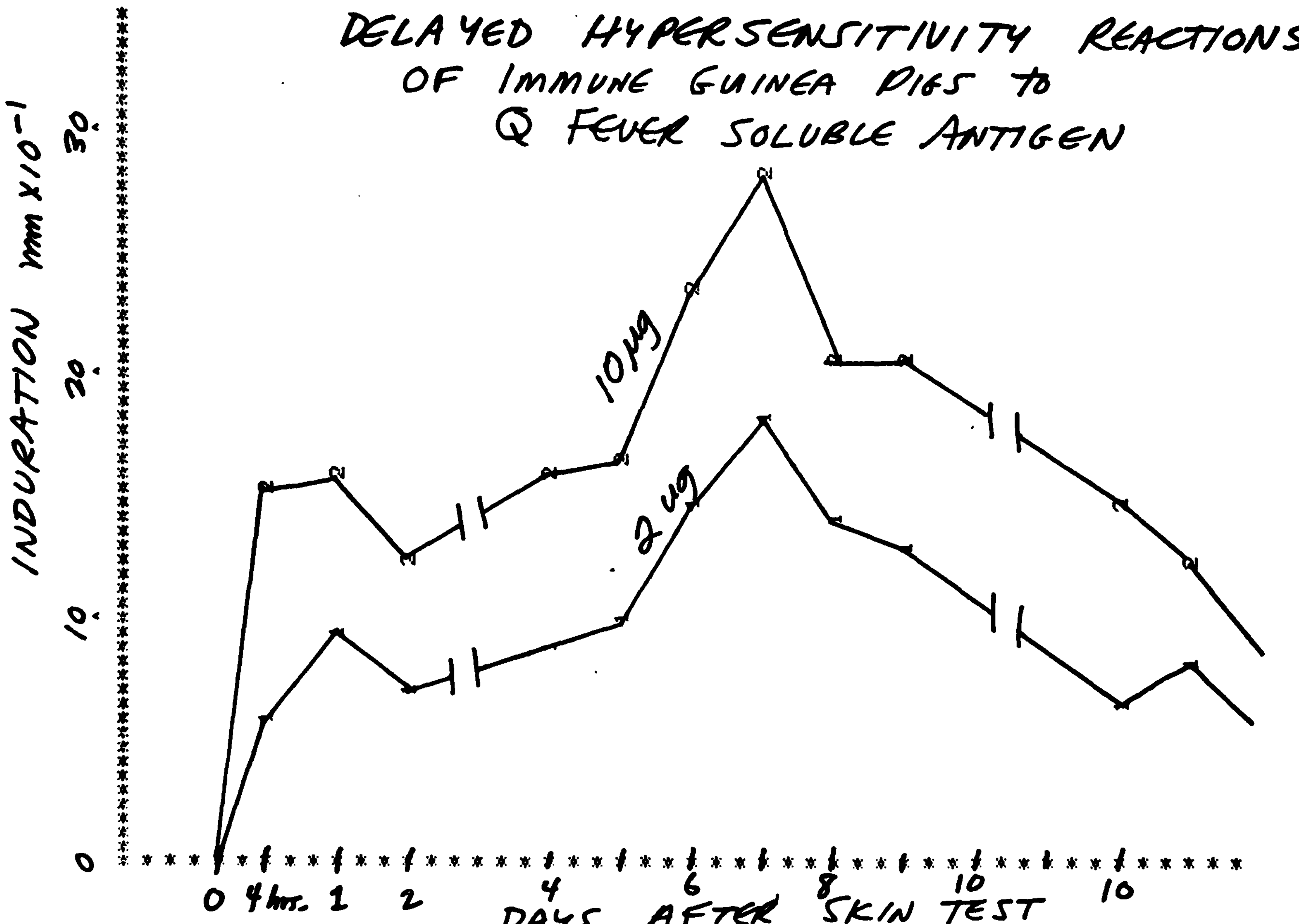


FIGURE 3


DELAYED HYPERSENSITIVITY REACTIONS
OF IMMUNE GUINEA PIGS TO
Q FEVER SOLUBLE ANTIGEN
INDURATION mm x10⁻¹
0
10
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30
10 μg
2 μg
0
4 hrs.
1
2
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10
10
DAYS AFTER SKIN TEST

Presented
RML Conference on Rickettsiae and
Rickettsial Diseases
Hamilton, MT
September 4, 1980

A Rapid Immunofluorescent Procedure for Serodiagnosis of Q Fever

Michael S. Ascher, James R. Greenwood, and Mary F. Thornton

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The true incidence of Q fever can only be estimated from retrospective surveys since diagnosis depends upon difficult serologic tests such as complement fixation or slide immunofluorescence usually performed only at reference laboratories. A new quantitative "dipstick" fluorescent antibody test has recently been perfected and we have utilized this technique and developed a rapid immunofluorescent assay for antibodies to Coxiella burnetti. The test takes a total of 1 hr to perform and employs stable reagents. We used as antigens two U.S. Army investigational vaccines, one in phase I and the other in phase II. The test involves air drying antigen on plastic sticks, incubation in serum and conjugate, and reading in a fluorometer.

We have tested sera from guinea pigs immunized with phase I vaccine and find phase I and phase II antibodies at titers in excess of 1:10,000 by this technique. We have also tested sera from individuals in our biosurveillance program and compared our results with standard phase II CF tests. Data will presented on the correlation between the two tests and on the sensitivity and reproducibility of the new method. Of interest, we note phase I antibody by this procedure in sera negative by phase I CF testing. Also, we have found that fluorescent-conjugated protein A will substitute for the anti-immunoglobulin in this assay, giving a selective assay for IgG.

In summary, we have developed a rapid quantitative immunofluorescent test for the serologic diagnosis of Q fever. This assay could easily be adapted for widespread use in the field by any diagnostic laboratory possessing a fluorometer. This test would seem to eliminate several major obstacles to rapid estimations of Q fever activity on a large scale.

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Annual meeting
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Federation Proceedings, 1981

DERMAL GRANULOMATOUS HYPERSENSITIVITY IN Q FEVER

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Q fever has been associated with granulomatous changes in clinical biopsy material obtained from liver and bone marrow. Local reactions to vaccine and skin testing have been described in previously sensitized humans but no histologic studies of such reactions have been reported. We note that delayed hypersensitivity reactions to whole cell phase I Q fever vaccine in immunized guinea pigs have a time course of development of induration characteristic of granulomatous hypersensitivity. Histology of such skin reactions on day 9 after testing reveals epithelioid cell infiltration and the presence of large numbers of multinucleated giant cells. Prominent in the sections are fragments of disintegrating polymorphonuclear leukocytes having the appearance of leukocytoclasis. Electronmicroscopic studies confirm the presence of epithelioid changes in cells of the mononuclear phagocyte series as well as extensive collagen deposition. Similar studies have been performed using TCA soluble and insoluble extracts and phase II vaccine as skin tests to determine the antigenic component responsible for granuloma formation. This animal system affords a readily reproducible model of dermal granulomatous hypersensitivity and an opportunity to analyze the immunologic basis of this reaction.
(Supported in part by the Burroughs-Wellcome Fund and USAMRDC)

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A RAPID IMMUNOFLUORESCENT PROCEDURE FOR SERODIAGNOSIS OF Q FEVER

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INTRODUCTION

Recent developments in technology have made it possible to accurately quantitate the results of fluorescent antibody procedures and eliminate much of the subjective component of such assays. These techniques have been successfully implemented on a commercial basis in the assay of antibody to rubella and anti-nuclear antibody(1,2), and additional antigenic systems are forthcoming from the manufacturer(3). The acronym for this procedure is FIAX. We have adapted this assay to the study of antibody in Q fever.

METHODS

In this procedure the carrier slide is replaced by a plastic "dipstick" coated on two sides with special paper. The assay instrument is a quantitative fluorometer specifically designed for this purpose. In these studies we have used as antigens two U.S. Army investigational Q Fever vaccines, one in Phase I and one in Phase II. The technical details involve placing 25 ul of antigen suspension on one

side of the stick and allowing it to air dry. The stick is then passed through tubes containing serum and conjugate along with intervening washes for a total assay time of one hour. The stick is then placed in the fluorometer chamber and each side is measured. The results appear in digital form on the instrument. The specific fluorescence of the sample (FSU) is defined as the result in arbitrary units of the antigen side less the result of the control side.

RESULTS

Table I shows the results of this assay applied to serum obtained from guinea pigs three weeks after immunization with 12 ug Phase I vaccine in Freund's complete adjuvwt. Results are presented for four serial dilutions of serum. Under these conditions, we note a marked difference between immune serum and control serum. These results were obtained after minimal manipulation of variables to obtain optimal concentrations of reagents.

TABLE I. FIAX Immunofluorescence assay of guinea pig serum antibodies to Phase I Q fever antigen

	Serum Dilution (reciprocal)			
Immunization	160	320	640	1280
None	2.4*	3.2	0.7	1.0
Phase I vaccine (12ug) + FCA	113.0	97.0	72.0	58.0

*FSU

A major question in the development of an immunologic assay for Q fever is the sensitivity of the test. Table II shows the antibody response of guinea pigs three weeks after immunization with varying amounts of phase I vaccine. The procedure detects a response in animals which received only 60 ng of antigen in the form of a skin test and shows a graded dose response from that form of immunization up to the Freund's complete adjuvant group. The data also illustrate the effect of varying the concentration of antigen on the sticks.

TABLE II. Effect of varying doses of immunizing vaccine on immunofluorescence of guinea pig sera with Phase I Q fever antigen.

Vaccine (ug)	Adjuvant	Antigen dilution (reciprocal) 32	16	8
None	None	8.9*	8.6	15.9
0.06	None	14.5	21.5	37.4
12.0	None	28.7	46.3	94.3
12.0	FCA	50.9	69.8	129.9

*FSU

In Table III we present the relationship between standard phase II complement fixation results and those obtained with the FIAX assay applied to human samples. The sera were obtained in the course of a biosurveillance program surrounding a recent outbreak of Q fever in a research laboratory.

TABLE III. Immunofluorescence assay of human serum antibody to Phase I and II Q fever antigens

Phase II CF Value	Phase II Antigen N	Mean FSU (SD)	Phase I Antigen N	Mean FSU (SD)
<8	68	26.9 (15.8)	34	0.1 (4.8)
8	11	41.1 (21.1)	11	3.0 (2.9)
16-32	12	76.1 (18.7)	5	11.5 (7.2)
$\geq$64	6	74.3 (19.7)	-	Not Done

The CF results are arbitrarily divided into four groups and the following observations can be made. One, all of the sera with clear positive results on CF, i.e. a titer of 1:16 or greater have FIAX values of 50 or more. Two, the sera wivh borderline CF titers of 8 center on the value of about 50 units. Three, sera negative by CF have a few results overlapping the positive range. In these few high grade reactors, we postulate that this assay is more sensitive than CF(4). One problem with these data is that the fluorescent values of the nonimmune human sera are considerably higher than those seen with sera from nonimmune guinea pigs. This noise in the assay has been reduced in other antigenic systems by the use of a tissue culture control antigen on the reverse surface of the stick and experiments of that type attempting to characterize this nonspecific background are in progress. Nevertheless, the overall performance of this assay to date suggests that it would serve as an excellent rapid screening procedure in the serodiagnosis of Q fever. In simple terms, any FIAX value of less than 50 can be assumed to be negative by CF and only those greater than 50 would be subjected to more cumbersome CF testing.

Finally, we tested this assay in man with phase I vaccine as antigen in addition to the phase II antigen. Table III demonstrates a similar degree of separation between CF positives and negatives but at a much lower level of response. We consider it significant that phase I reactivity can be detected in these individuals and this finding will be pursued in recipients of the new phase I vaccine.

SUMMARY

We have used a new simplified FIAX immunofluorescent procedure to demonstrate antibody to C. burnetii in guinea pigs and man. The test is extremely sensitive and correlates very well with standard Phase II CF serology. The widespread dissemination of FIAX instruments to clinical laboratories should make it possible for more facilities to perform their own screening tests for Q fever and may relieve the burden placed on reference laboratories by biosurveillance of sheep research programs.

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REFERENCES

1. Brody, J.P., Binkley, J.H., and Harding, S.A. (1979) J Clin Microbiol 10, 708.
2. Ross, G.L., Barland, P., and Grayzel, A.I. (1978) J Rheum 5, 373.
3. Walls, K.W., and Barnhart, E.R. (1978) J Clin Microbiol 7, 234.
4. Kazar, J., Brezina, R., Kovacova, E., and Urvolgyi, J. (1973) Acta Virol 17,512.

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DRAFT

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The true incidence of Q fever can often be estimated only from retrospective surveys since diagnosis depends upon difficult serologic tests such as complement fixation or slide immunofluorescence usually performed only at reference laboratories. A new quantitative "dipstick" fluorescent antibody test (FIAX) has recently been perfected for measuring antibodies in several microbial systems. We have utilized this technique and developed a rapid immunofluorescent assay for antibodies to Coxiella burnetii. The test takes a total of 1 hr to perform and employs stable reagents. We used as antigens two investigational Q fever vaccines, one in phase I and the other in phase II. The test involves air drying antigen on plastic sticks, incubation in serum and conjugate, and reading in a fluorometer.

We have tested sera from guinea pigs immunized with phase I vaccine and find phase I and phase II antibodies at titers in excess of 1:10,000 by this technique. We have also tested sera from individuals in a biosurveillance program and compared our results with standard phase II CF tests. There is excellent correlation between the two tests and the sensitivity and reproducibility of the new method is comparable to or better than CF. We have also found that fluorescent-conjugated protein A will substitute for the anti-immunoglobulin in this assay, giving a selective assay for IgG which can be used in experimental animals as well as man.

Recent developments in technology have made it possible to accurately quantitate the results of fluorescent antibody procedures and eliminate much of the subjective component of such assays. These techniques have been successfully implemented on a commercial basis in the assay of antibody to rubella (3002) and anti-nuclear antibody, or ANA (3003,3001), and additional antigenic systems have been used by individual laboratories (3004). The acronym for this procedure is FIAX. We have adapted this assay to the study of antibody in Q fever.

MATERIALS AND METHODS

Guinea pigs. Outbred Hartley guinea pigs were used and maintained as previously described.

Vaccines. Phase I and phase II

Immunizations. Guinea pigs were immunized with vaccine emulsified in FCA or neat at 0.4 ml of total suspension (0.1 ml/footpad).

FIAX procedure. Standard FIAX procedures (   ) were used with the exception of a goat antiguinea pig antiserum and FITC-conjugated protein A obtained from TAGO.

In brief, sticks were coated with 50 ul of a 1:8 dilution of vaccine for a final dose of   ug (Phase I) and a 1:100 dilution for a final dose of   ug (Phase II). MARY

RESULTS

Figure 1 shows the results of this assay applied to serum obtained from guinea pigs immunized with Phase I Q fever vaccine in Freund adjuvant. The response in flurometric units is depicted on the ordinate and the serum dilution is displayed on the abcissa. Under these conditions, we note a striking response with immune serum compared to control serum. This result was obtained after minimal manipulation of variables to obtain optimal concentrations of reagents. Figure 2 shows the antibody response of guinea

pigs immunized with decreasing amounts of phase I vaccine. The procedure detects a response in animals which received 60 ng of antigen in the skin test only group and shows a graded dose response from that form of immunization up to the Freund adjuvant group. The group called "Q" in the figure received 12 ug of vaccine without adjuvant, a two hundred fold greater dose than the skin test.

One of the difficulties associated with immunoassays in general is the reproducibility and stability of reagents. In fluorescent antibody techniques the most variable component of the reaction sequence is generally the anti-antibody conjugated with fluorescein tag. Borrowing from prior work with RIA (Jahrling), we examined the possibility that a chemically defined carrier with specificity for immunoglubulin, staphylococcal protein A, might substitute for specific antiserum. We note in figure 3 that protein A-FITC produces equivalent results as an anti-antibody when compared to those with goat antiserum in the first data slide. Most importantly, protein A has specificity for IgG and when used in combination with selective antisera should give additional information as to the class of antibody produced in response to Q fever vaccine in forthcoming studies.

In figure 4 we illustrate the relationship between standard phase II complement fixation results and those obtained with the FIAX assay applied to human samples. The sera in this figure were obtained in the course of a biosurveillance program surrounding a recent outbreak of Q fever in the laboratory. If the CF results are arbitrarily divided into four groups as noted, the following observations can be made. One, all of the sera with clear positive results on CF, i.e. a titer of 1:16 or greater have FIAX values of 50 or more. Two, the sera with borderline CF titers of 8 center on the value of about 50 units. Three, sera negative by CF have a few results overlapping the positive range. In the few high grade reactors by

FIAX who are negative by CF, we postulate that this assay is more sensitive than CF. One problem with these data is that the fluorescent values of the nonimmune human sera is considerably higher than those found in guinea pigs. This noise in the assay has been reduced in other antigenic systems by the use of a tissue culture control antigen on the reverse surface of the stick and experiments of that type attempting to characterize this nonspecific background are in progress. The overall performance of this test to date suggest that it would serve as an excellent rapid screening procedure in the serodiagnosis of Q fever. In simple terms, any FIAX value of less than 50 can be assumed to be negative by CF and only those greater than 50 would be subjected to more cumbersome CF testing.

In Figure 5, the results of FIAX assay of human sera with phase I vaccine as antigen in addition to the phase II results just shown. This figure demonstrates a similar degree of separation between CF positives and negatives but at a lower level of response. We consider it significant that phase I reactivity can be detected in these individuals and this finding will be pursued in recipients of the new phase I vaccine.

Our human serum specimens include thirty three (33) ~~fifteen~~ pairs of samples obtained 3 months apart. Thirty (30) ~~Twelve~~ of the ~~fifteen~~ pairs were from uninfected individuals with unchanging CF values. Three of the pairs were from patients ill with Q fever. Figure 6 depicts the serial observations in these pairs. We note the wide range of FIAX values for a given CF value as previously, but note a remarkable stability of this assay in the uninfected individuals. The infected individuals show a clear change in FIAX activity after Q fever.

3001 Casavant, C.H., A.C. Hart, and D.P. Stites. Comparison of a semiautomated fluorescent immunoassay system and indirect immunofluorescence for detection of antinuclear antibodies in human serum. J Clin Microbiol 10:712-718, 1979.

3002 Brody, J.P., J.H. Binkley, and S.A. Harding. Evaluation and comparison of two assays for detection of immunity to rubella infection. J Clin Microbiol 10:708-711, 1979.

3003 Ross, G.L., P. Barland, and A.I. Grayzel. The immunoglobulin class of anti-DNA antibodies: Detection by a fluorometric immunoassay: Clinical and pathological correlations in SLE. J Rheum 5:373-383, 1978.

3004 Walls, K.W. and E.R. Barnhart. Titration of human serum antibodies to <u>Toxoplasma</u> <u>gondii</u> with a simple fluorometric assay. J Clin Microbiol 7:234-235, 1978.

✓ NEED FIAX on FECAL BLOOD LOSS REFERENCE

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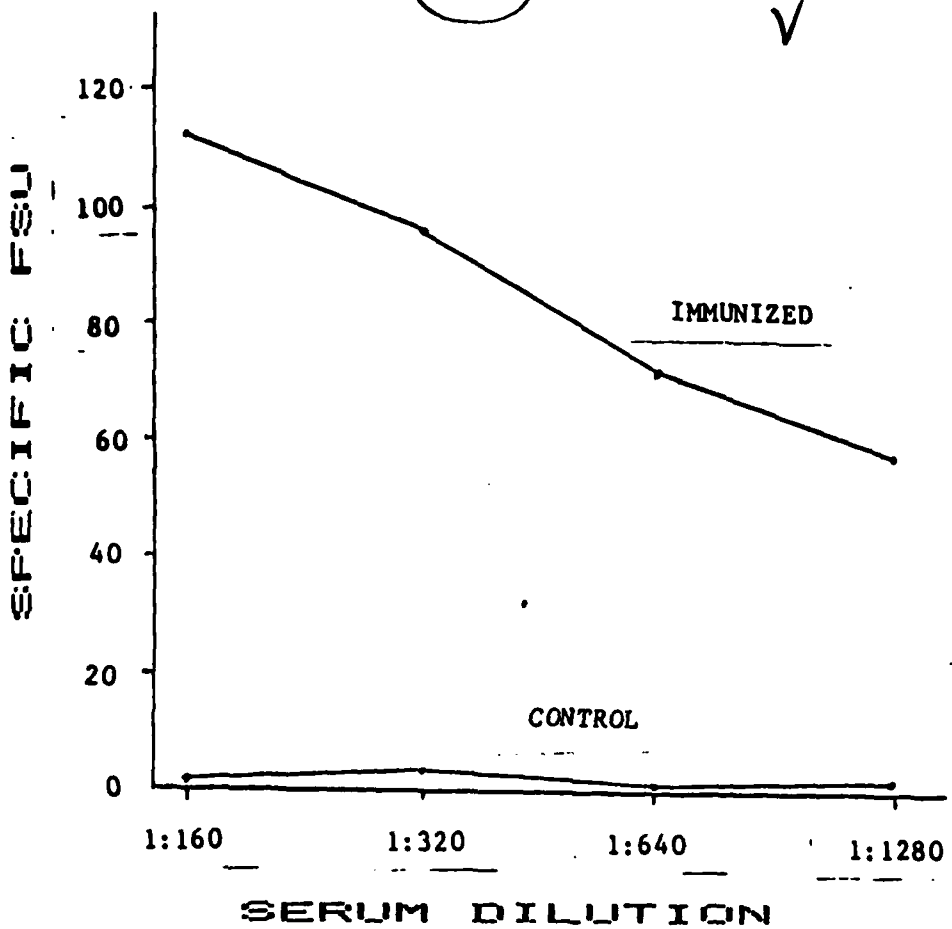
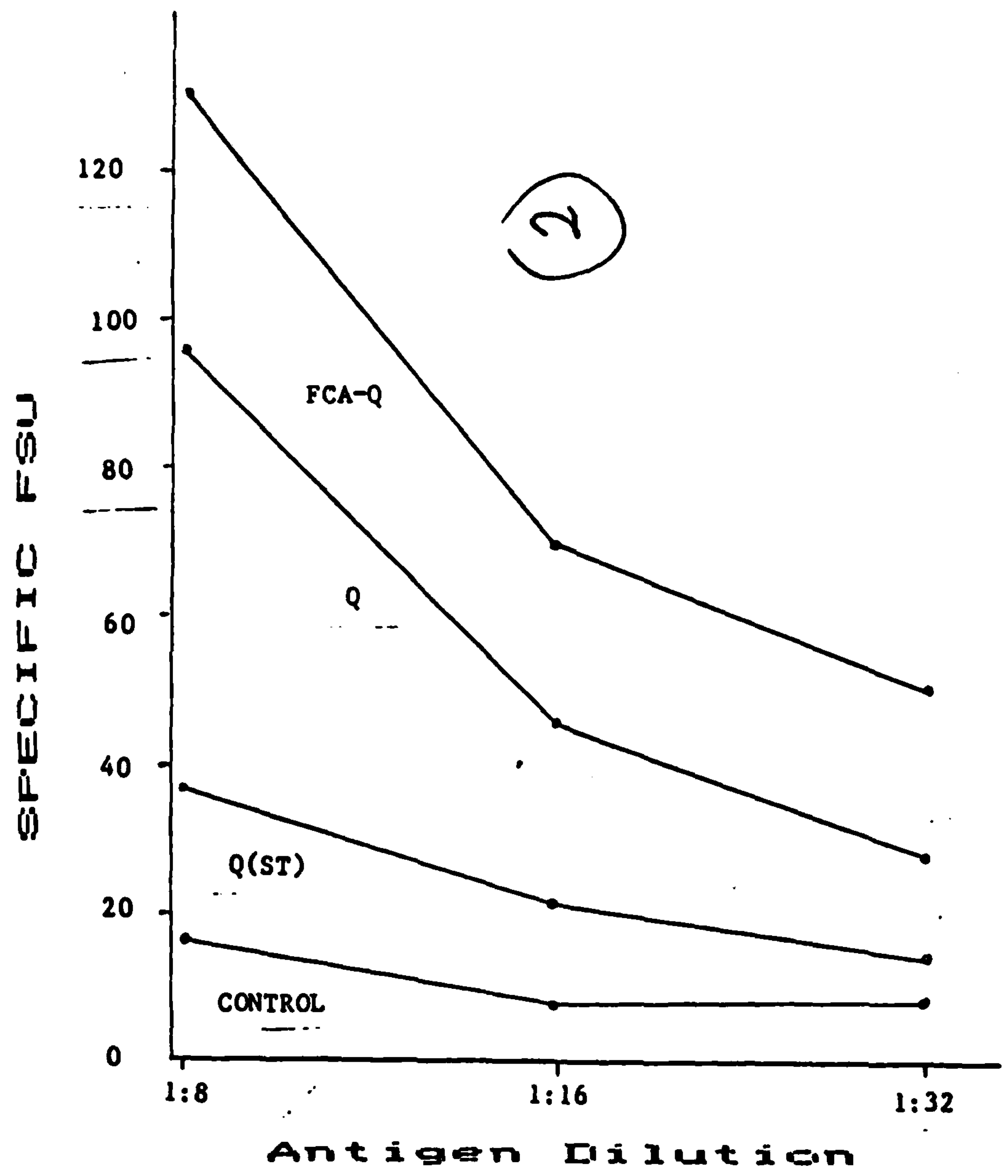
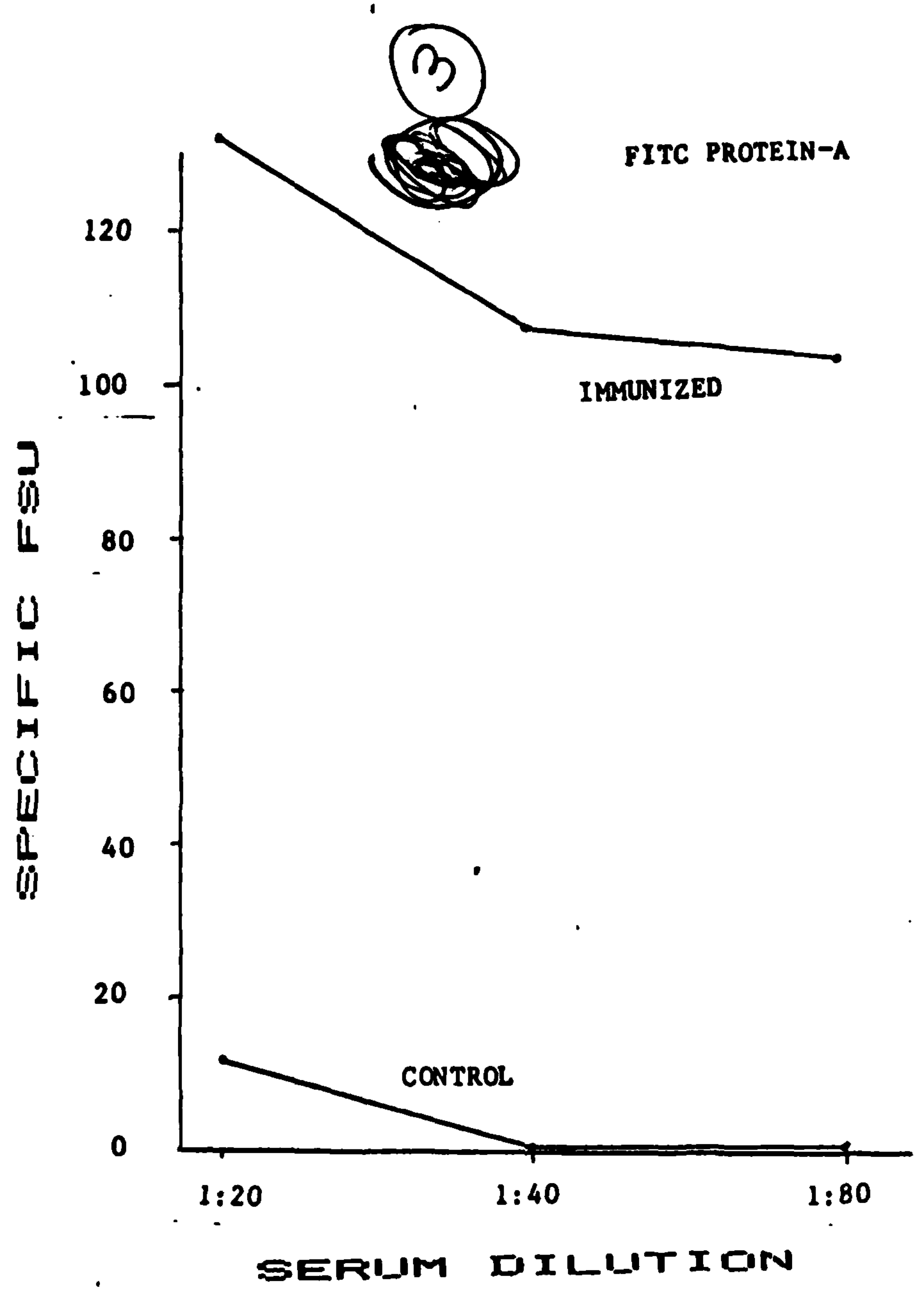
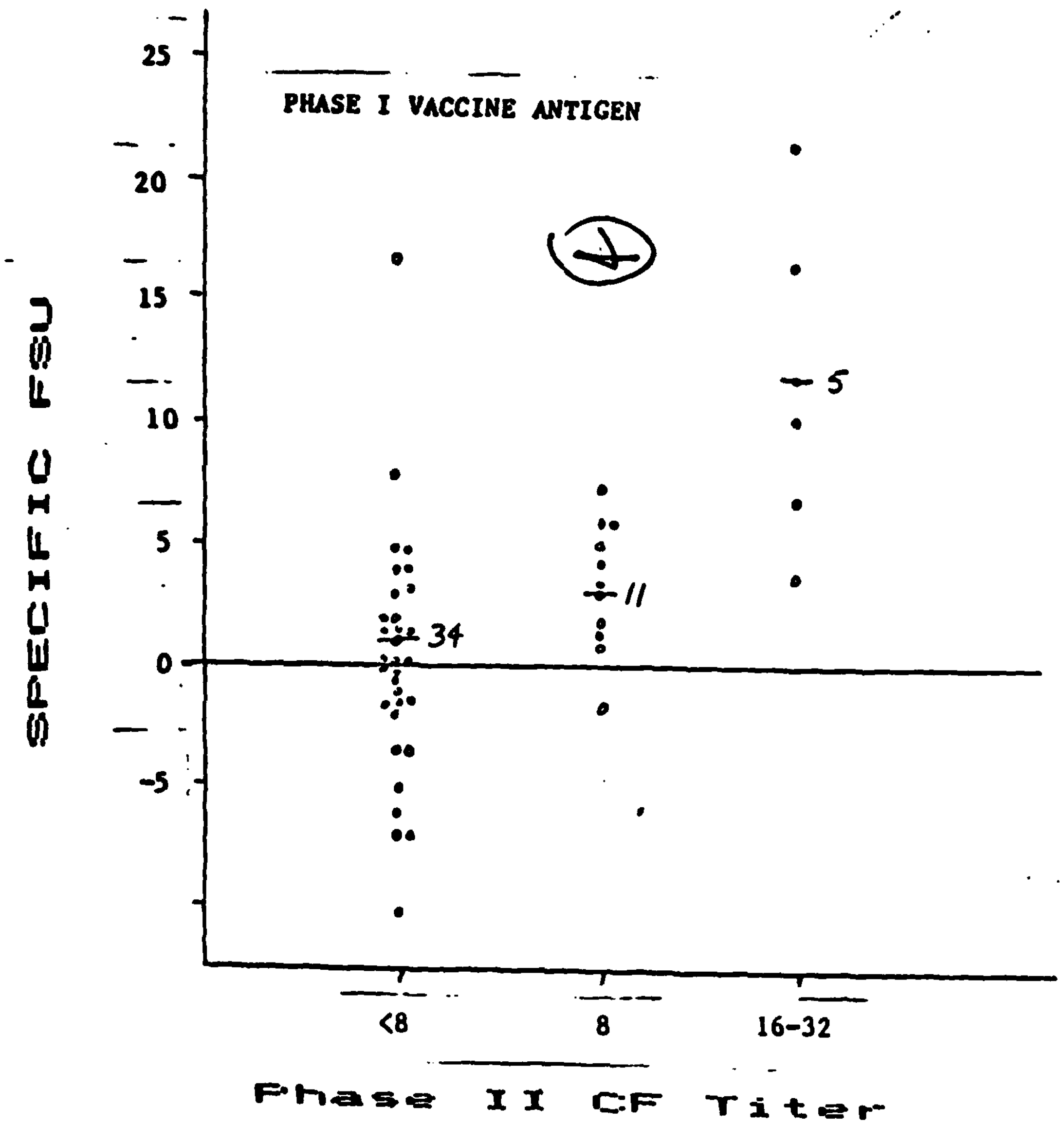


SPECIFIC FSU
120
100
80
60
40
20
0
IMMUNIZED
CONTROL
1:160
1:320
1:640
1:1280
SERUM DILUTION

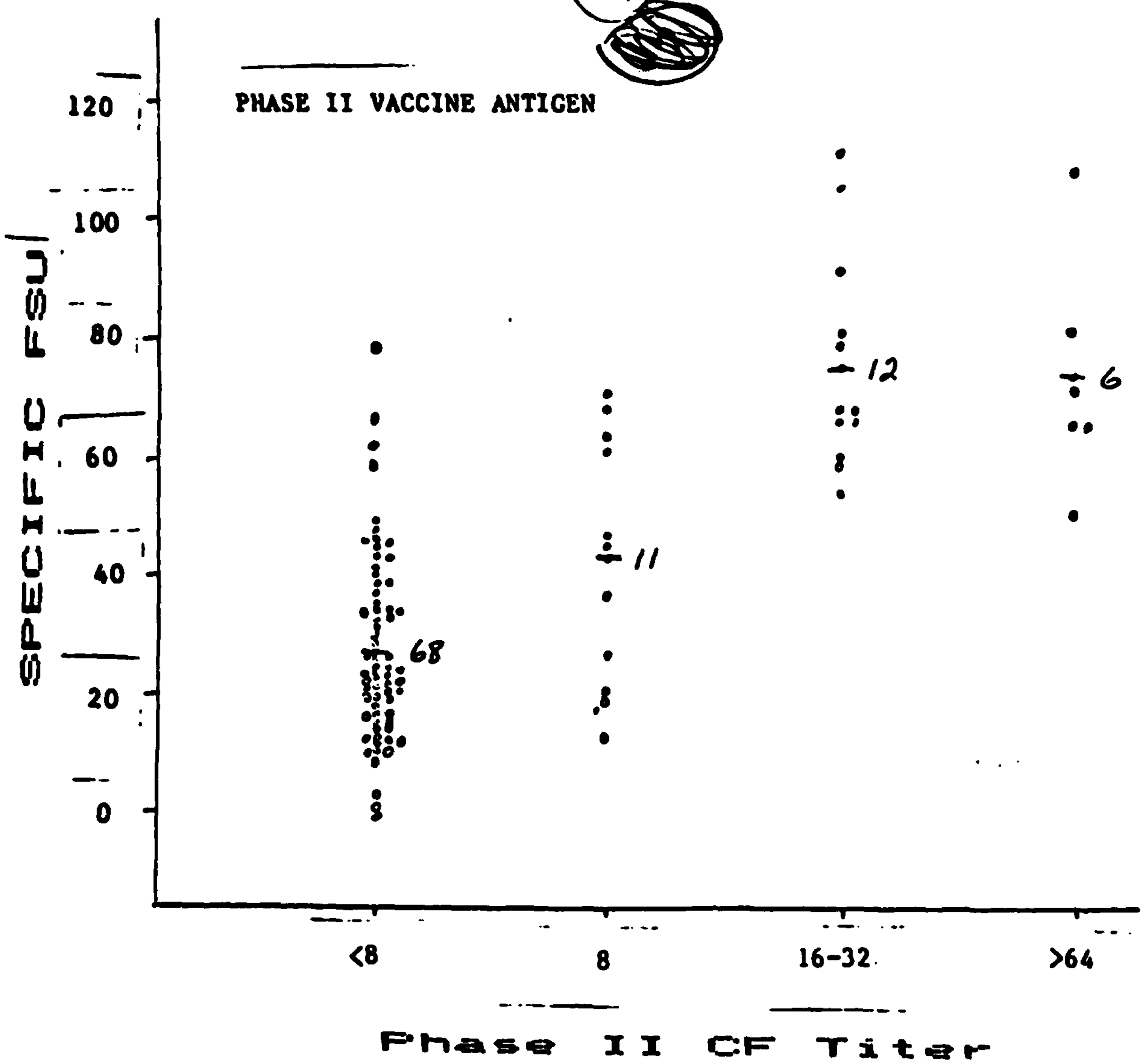


(2)
SPECIFIC FSU
120
100
80
60
40
20
0
FCA-Q
Q
Q(ST)
CONTROL
1:8
1:16
1:32
Antigen Dilution


3
FITC PROTEIN-A
IMMUNIZED
CONTROL
SPECIFIC FSU
120
100
80
60
40
20
0
1:20
1:40
1:80
SERUM DILUTION

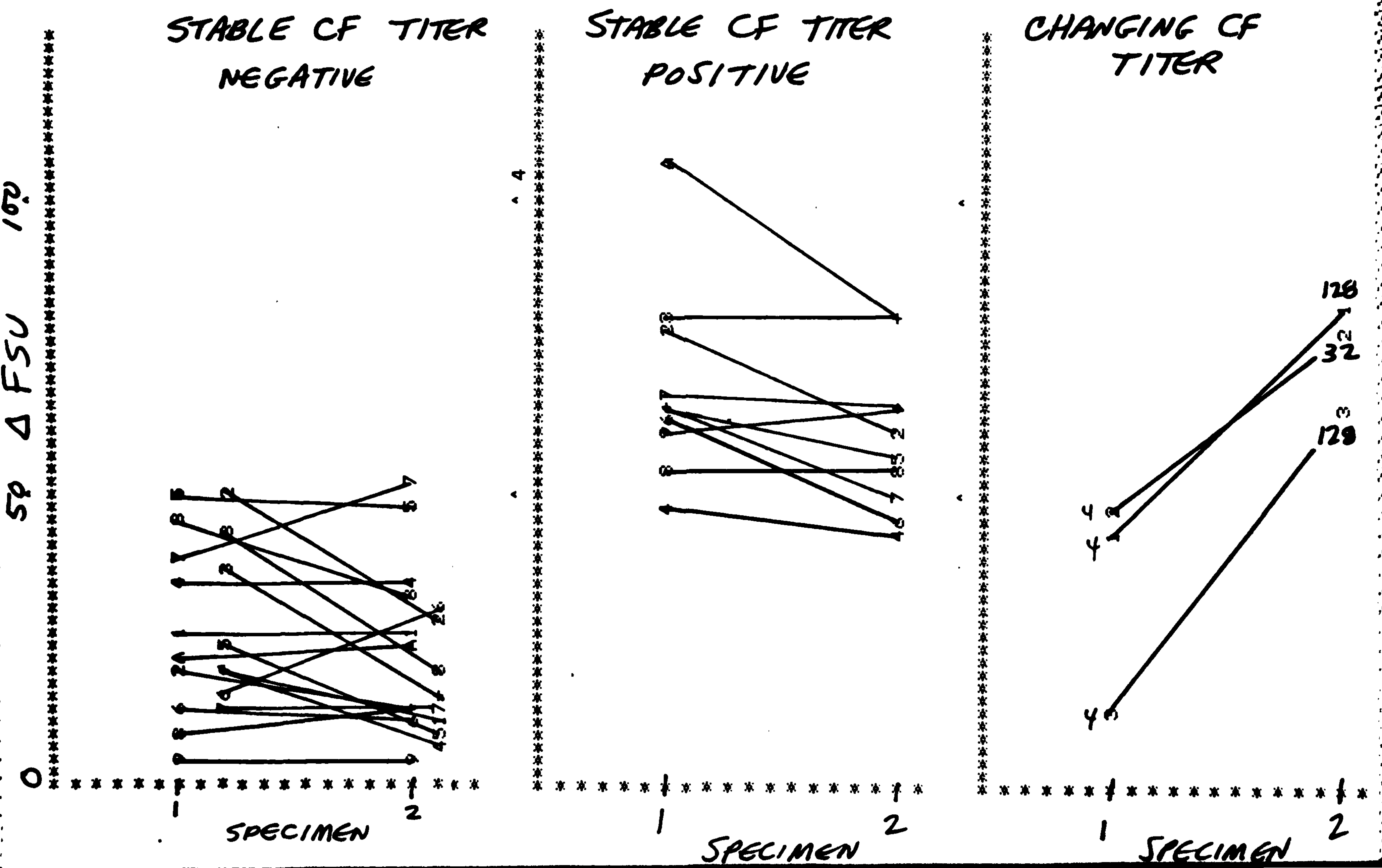


PHASE I VACCINE ANTIGEN
4
SPECIFIC FSU
25
20
15
10
5
0
-5
34
11
5
<8
8
16-32
Phase II CF Titer



PHASE II VACCINE ANTIGEN
SPECIFIC FSU
0
20
40
60
80
100
120
68
11
12
6
<8
8
16-32
>64
Phase II CF Titer

FIGURE 6 - SERIAL FIAX VALUES ON HUMAN SERA



MS 109 (,13)

DERMAL GRANULOMATOUS HYPERSENSITIVITY IN Q FEVER

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DRAFT

ABSTRACT

Q fever has been associated with granulomatous changes in clinical biopsy material obtained from liver and bone marrow. Local reactions to vaccine and skin testing have been described in previously sensitized humans but no histologic studies of such reactions have been reported. We note that delayed hypersensitivity reactions to whole cell phase I Q fever vaccine in immunized guinea pigs have a time course of development of induration characteristic of granulomatous hypersensitivity. Histology of such skin reactions on day 9 after testing reveals epithelioid cell infiltration and the presence of large numbers of multinucleated giant cells. Prominent in the sections are fragments of disintegrating polymorphonuclear leukocytes having the appearance of leukocytoclasis. Electronmicroscopic studies confirm the presence of epithelioid changes in cells of the mononuclear phagocyte series as well as extensive collagen deposition. Similar studies have been performed using TCA soluble and insoluble extracts and phase II vaccine as skin tests to determine the antigenic component responsible for granuloma formation. This animal system affords a readily reproducible model of dermal granulomatous hypersensitivity and an opportunity to analyze the immunologic basis of this reaction.

INTRODUCTION

Q fever is an enzootic infection of sheep and cattle passed to man through direct or indirect contact. The clinical illness in man is usually an undifferentiated fever, but atypical pneumonia and hepatitis are frequently found when appropriate examinations are performed. The mortality is very low in the uncomplicated infection, but a chronic infectious state has been described rarely in man with destruction of heart valves and high mortality (1206, 1257).

Since the initial description of the histology of Q fever in mice (1303), numerous reports have noted granulomatous changes in liver and bone marrow samples obtained from animals and patients (book,1289,1304,1300,1302,1248,1252,1195,1216,1220). Variable degrees of giant cell formation have been described and blood vessel involvement was claimed in one early report (1302). Subsequent workers were unable to demonstrate angiitis, but did note a characteristic appearance of the granuloma; a "doughnut" of epithelial cells around a central adipocyte (1248,1252). On the one occasion where liver biospy material was adequately examined for the presence of rickettsiae, they were found to be present (1195).

In parallel with the clinical and histological descriptions noted above, several sets of workers have studied the effects of Q fever vaccines in man and experimental animals. One repetitive finding in these studies is the development of adverse local reactions in a proportion of recipients (1301,1209,1226,1199,1006). Skin tests have been performed in a number of studies in an attempt to detect pre-existing sensitivity and avoid such vaccine reactions. Two types of skin reactions have been described macroscopically, but no histologic studies have been reported.

We report herein studies on the histology of dermal reactivity in

sensitized guinea pigs to Q fever vaccine.

MATERIALS AND METHODS

Guinea pigs. Outbred Hartley strain guinea pigs of either sex weighing 350-500 g were used. The animals came from stocks held at the Royal College of Surgeons or were purchased from A. Tuck & Son, Ltd., Battlesbridge, Essex, or David Hall, Newchurch, Staffs. They were fed on pelleted diet RGP (F. Dixon & Son, Ware, Herts), liberally supplemented with cabbage and hay.

Q fever vaccine. The Q fever vaccine is an investigational product (IND #610) prepared by Merrell-National Laboratories and kindly supplied by the U.S. Army Medical Research Institute of Infectious Diseases for these studies.

Immunizations. Guinea pigs were injected with Q fever vaccine emulsified in Freund complete adjuvant at a final concentration of 30 ug/ml rickettsiae protein. A total of 0.4 ml (12 ug) was injected into the four footpads of the guinea pigs.

Delayed hypersensitivity testing. On day 21 after sensitization, the guinea pigs were injected in the shaved flank with 0.1 ml of a 1:100 dilution of vaccine for a skin test dose of 0.06 ug. The reactions were scored for intensity and diameter of erythema and increase in skin thickness determined with a skin calliper as previously described (1020). In these studies measurements were made at 4 and 24 hours and daily thereafter for up to 21 days.

Histologic examination of tissues. Skin test reaction sites were fixed in Bouin's solution, sectioned at 5 u and stained with hematoxylin and eosin.

Electron microscopic procedures.

RESULTS

The result of injecting Q fever vaccine intradermally into the skin of a previously sensitized guinea pig is grossly delayed hypersensitivity until day 3. In contrast to usual DH, there then develops at the skin test site a 5-8 mm nodule without erythema but with some superficial flaking of the epidermis over the site. The quantitative representation of this reaction is shown in Figure 1 where the course of increase in skin thickness change is plotted versus time. The reaction of unimmunized guinea pigs shown in the figure is similar to that of immunized animals over the first 24 hours.

Figure 2 illustrates the presence in the reaction of a giant cell and disintegrating fragments of nuclei of polymorphonuclear leukocytes, so-called "leukocytoclastic changes" (book). Figure 3 shows the electronmicroscopic appearance of cells of the mononuclear phagocyte system (MPS) making up the infiltrate of the Q fever skin test. The cell in the field illustrated is typical epitheloid cell (1294) with pale oval nucleus, prominent nucleolus and rather empty cytoplasm with swollen rough endoplasmic reticulum. There was no suggestion of macrophage phagocytic activity in the section examined and no rickettsial bodies wre identified. Figure 4 illustrates the ultrastructure of a multinucleated giant cell seen in this reaction. Cells of fibroblast morphologic appearance were prominent in these sections and in Figure 4 we also note bundles of collagen.

DISCUSSION

In previous studies with Q fever vaccine, cutaneous reactivity in man has been noted frequently (1301). There are several reports that skin reactions have typical features of "delayed hypersensitivity", but a late component has also been frequently noted. In one study it was claimed that the late reaction was a feature of "active immunity associated with local antibody production" (1199). Our analysis shows a virtual identity grossly between the skin thickness change data with Q fever vaccine and those previously described with zirconium lactate in sensitized guinea pigs (1296). The Q fever lesions, however, are more intense and reproducible in our hands.

The light microscopic histologic changes in the Q fever reactions are those of epithelioid granulomas with giant cell formation. We note also the presence of leukocytoclastic changes which have not been previously noted in the guinea pig. The electronmicroscopic findings are those of epithelioid changes in cells of the MPS (1294,1295,1299,1298). These cells are virtually identical with those reported previously in NaZr L granulomas in the guinea pig, and recently reported for BCG in guinea pig lymph nodes. The difference, however, is the fact that the Q fever vaccine generates dermal granulomas, a model which heretofore was lacking. This easily reproducible technique may be useful in analysis of the granulomatous immunologic basis of hypersensitivity. Additionally, and most significant to the Q fever problem, this technique provides an assay for determining the antigenic component of the organisms responsible for the granulomatous reaction and may allow us to develop a purified nongranulomogenic reagent for diagnosis of prior sensitivity to Q fever in man.

REFERENCES

1192 Anacker, R.L., D.B. Lackman, E.G. Pickens, and E. Ribi. 1962. Antigenic and skin-reactive properties of fractions of Coxiella burnetii. J Immunol 89:145-153.

1020 Ascher, M.S., D. Parker, and J.L. Turk. 1977. Modulation of delayed-type hypersensitivity and cellular immunity to microbial vaccines: Effects of cyclophosphamide on the immune response to tularemia vaccine. Infect Immun 18:318-323.

1226 Bell, J.F., L. Luoto, M. Casey, and D.B. Lackman. 1964. Serologic and skin-test response after Q fever vaccination by the intracutaneous route. J Immunol 93:403-408.

1220 Bernstein, M., H.A. Edmondson, and B.H. Barbour. 1965. The liver lesion in Q fever. Arch Intern Med 116:491-498.

1248 Delsol, G., M. Pellegrin, J. Familiades, and J.C. Auvergnat. 1978. Bone marrow lesions in Q fever. Blood 52:637-638 (letter).

1195 DuPont, H.L., R.B. Hornick, H.S. Levin, M.I. Rapoport, and T.E. Woodward. 1971. Q fever hepatitis. Ann Intern Med 74:198-206.

1302 Ende, N. and A.P. Gelpi. 1957. Pathological changes noted in bone marrow in a case of Q fever. Arch Int Med 100:793-796.

1206 Ferguson, I.C., J.E. Craik, and N.R. Grist. 1962. Clinical, virological, and pathological findings in a fatal case of Q fever endocarditis. Brit J Clin Path 15:235-241.

1300 Gerstl, B., E.R. Movitt, and J.R. Skahen. 1956. Liver function and morphology in "Q" fever. Gastroenterology 30:813-819.

1199 Lackman, D.B., E.J. Bell, J.F. Bell, and E.G. Pickens. 1962. Intradermal sensitivity testing in man with a purified vaccine for Q fever. Am J Pub Health 52:87-93.

1301 Lackman, D.B., J.F. Bell, C.L. Larson, M.L. Casey, and W.W. Benson.

1959. An intradermal sensitivity test for Q fever in man. Arch Inst Pasteur (Tunis) 36:557-569.

BOOK Histopathlogy of the Skin, 5th Edition. Lever, W.F. and G.S. Lever, eds. Lippincott, Philadelphia and Toronto, 1975, pp. 160-167.

1305 Lillie, R.D. 1942. Pathologic Histology in guinea pigs following intraperitoneal inoculation with the virus of "Q" fever. Pub Health Rep 57:296-306.

1304 Lillie, R.D., T.L. Perrin, and C. Armstrong. 1941. An institutional outbreak of pneumonitis. III. Histopathology in man and rhesus monkeys in the pneumonitis due to the virus of "Q" fever. Pub Health Rep 56:149-155.

1209 Luoto, L., J.F. Bell, M. Casey and D.B. Lackman. 1963. Q fever vaccination of human volunteers. I. The serologic and skin-test response following subcutaneous injections. 1963. Am J Hyg 78:1-15.

1294 Narayanan, R.B., P. Badenoch-Jones, and J.L. Turk. Experimental mycobacterial granulomas in guinea pig lymph nodes: Ultrastructural observations. J Path (submitted).

1252 Okun, D.B., N.C.J. Sun, and K.R. Tanaka. 1979. Bone marrow granulomas in Q fever. Am J Clin Path 71:117-121.

1006 Peacock, M.G., P. Fiset, R.A. Ormsbee, and C.L. Wisseman, Jr. 1979. Antibody response in man following a small intradermal inoculation with Coxiella burnetii phase I vaccine. Acta Virol 23:73-81.

1303 Perrin, T.L. and I.A. Bengtson. 1942. The histopathology of experimental "Q" fever in mice. Pub Health Rep 57:790-798.

1216 Picchi, J., A.R. Nelson, E.E. Waller, M. Razavi, and E.E. Clizer. 1960. Q fever associated with granulomatous hepatitis. 53:1065-1074.

1298 Ridley, M.J., P. Badenoch-Jones, and J.L. Turk. 1980. Ultrastructure

of cells of the mononuclear-phagocyte series (MPS) across the leprosy spectrum. J Path 130:223-227.

1299 Turk, J.L., P. Badenoch-Jones, and D. Parker. 1978. Ultrastructural observations on epithelioid cell granulomas induced by zirconium in the guinea-pig. J Path 124:45-49.

1257 Turck, W.P.G., G. Howitt, L.A. Turnberg, H. Fox, M. Longson, M.B. Matthews, and R. Das Gupta. 1976. Chronic Q fever. Quart J Med (New Series) 178:193-217.

1297 Turk, J.L. 1980. Immunologic and nonimmunologic activation of macrophages. J Invest Derm 74:301-306.

1295 Turk, J.L. and D. Parker. 1977. Granuloma formation in normal guinea pigs injected intradermally with aluminum and zirconium compounds. J Invest Derm 68:336-340.

1296 Turk, J.L. and D. Parker. 1977. Sensitization with Cr, Ni, and Zr salts and allergic type granuloma formation in the guinea pig. J Invest Derm 68:341-345.

FIGURE 1

DELAYED HYPERSENSITIVITY REACTIONS OF IMMUNIZED GUINEA PIGS TO Q FEVER VACCINE

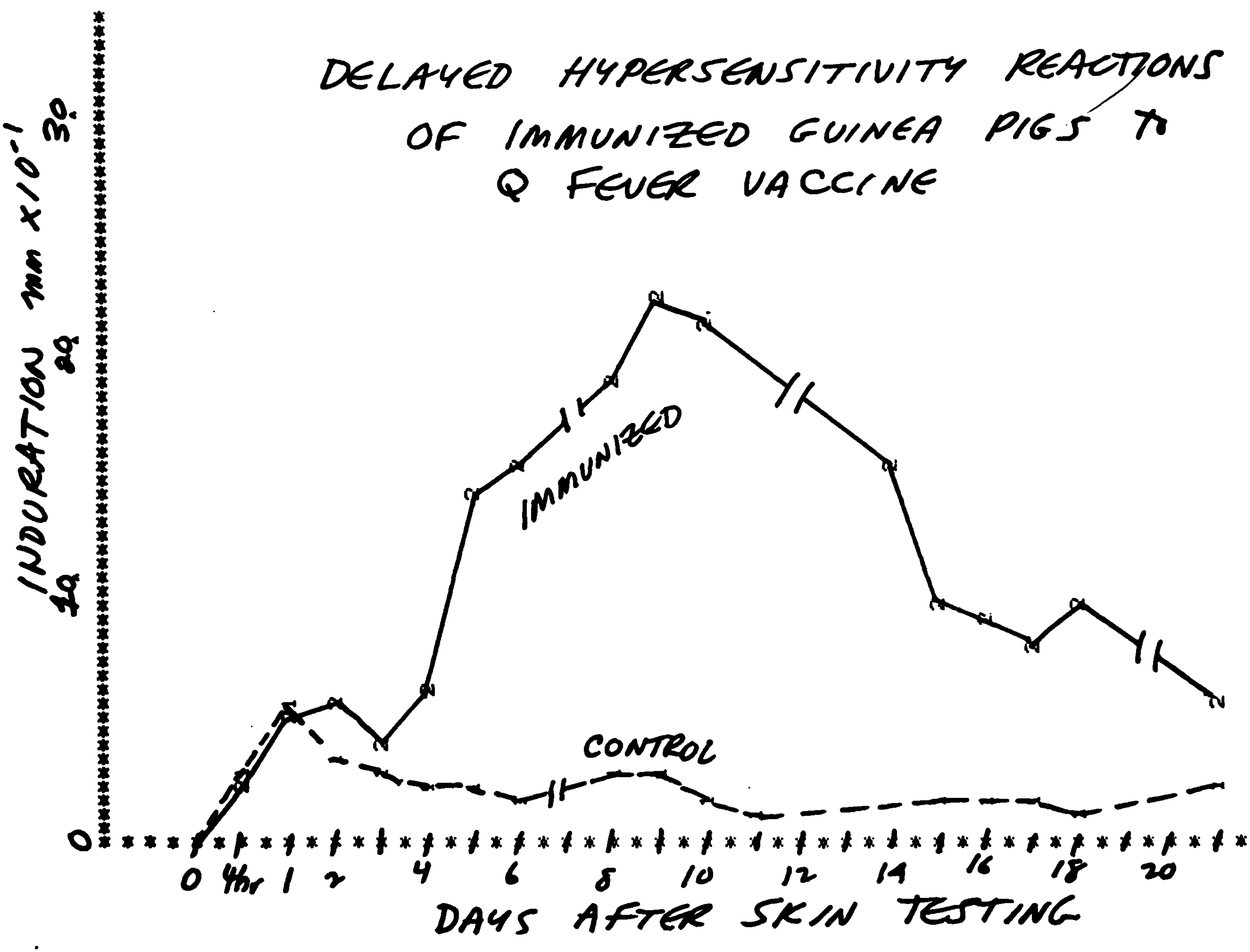


Figure 2

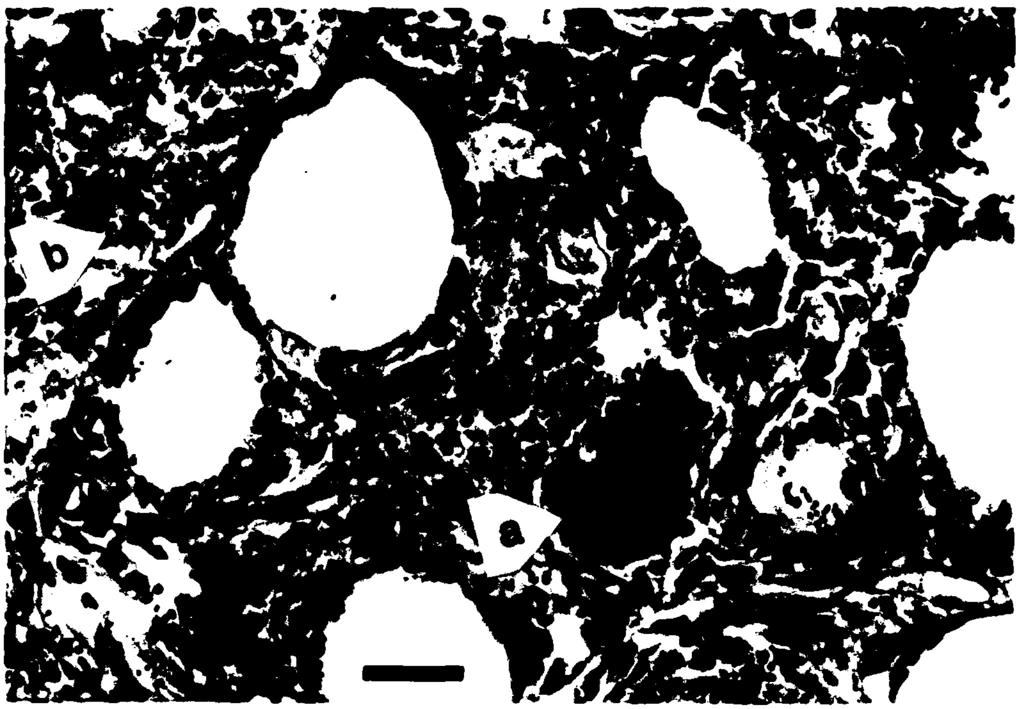


Figure 3

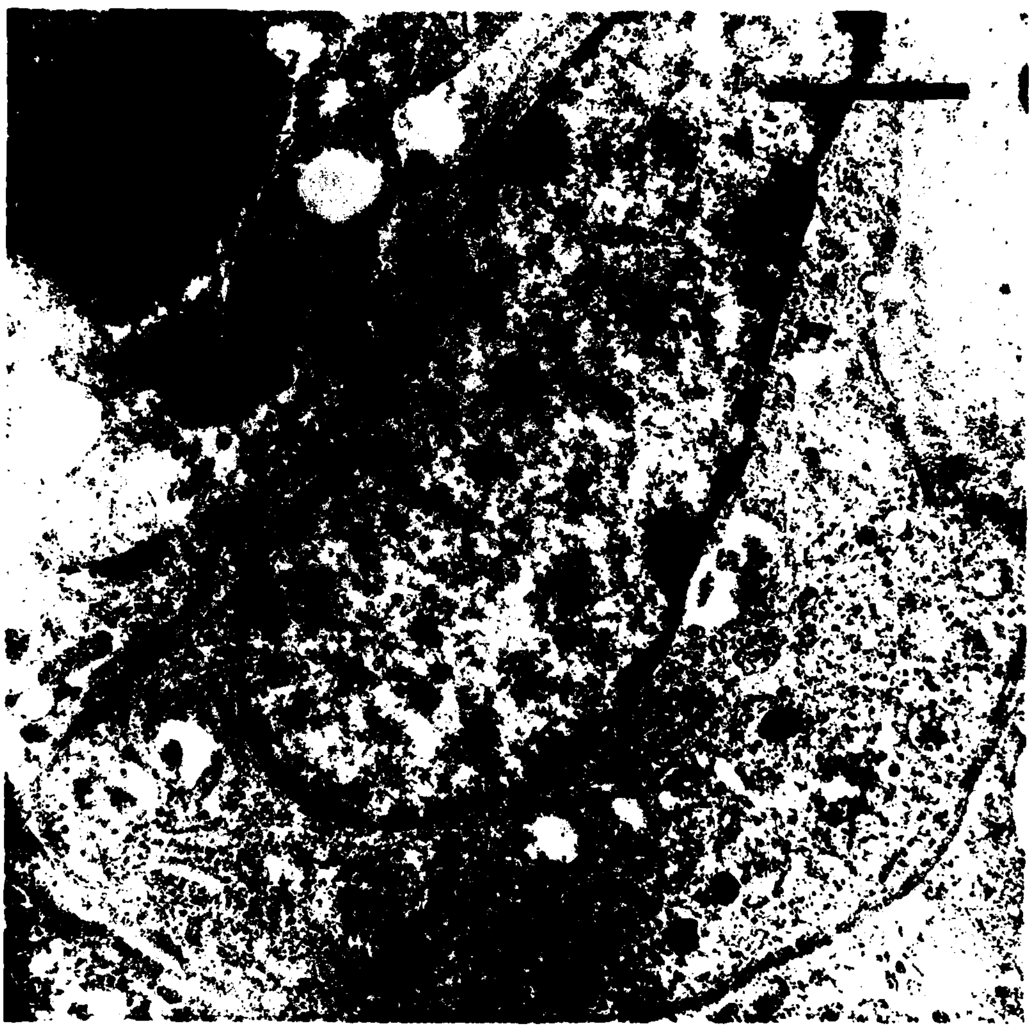
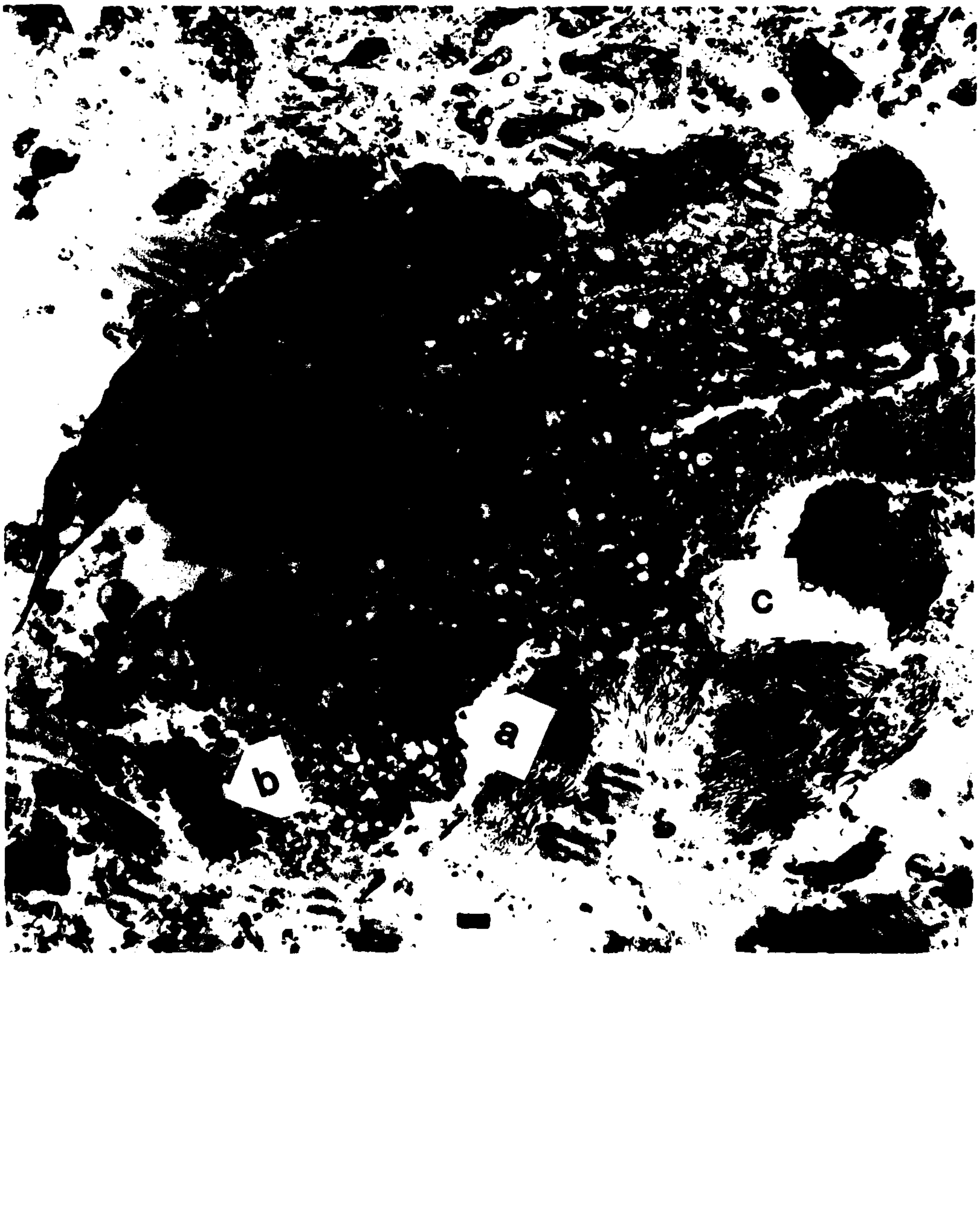


Figure 4



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